

## Supplementary Materials

**Fig. S1. Cell clonality and stability analysis of the PEM-R cells.** (A) Colony formation assay was performed using A549 and A549/PEM cells that were treated using PEM or DMSO as the control for 2 weeks, with the results evaluated using analysis of variance ( $n = 5$ ). (B) The two PEM-R cell lines were allowed to grow or remain in culture for 8 weeks after thawing, and the resistance indexes were presented ( $n = 3$ ). (C) The growth rates of PEM-R cells and their parental cells were calculated by counting the numbers of cells from day 1 to day 5, with the results evaluated using analysis of variance ( $n = 3$ ). NS: not statistically significant, \*\*\* $P < 0.001$ .

**Fig. S2. The distribution of UCHL1 protein in NSCLC cells.** The ratio of the intranuclear UCHL1 fluorescence intensity to the total UCHL1 fluorescence intensity in H1299 cells, H1299/PEM cells, A549 cells, and A549/PEM cells was shown and evaluated using the Mann-Whitney test ( $n = 3$ ). NS: not statistically significant.

**Fig. S3. Colony forming efficiency and the role of LDN57444 in the survival of NSCLC cells.** (A) The ubiquitin protein levels in H1299/PEM cells after treatment for 48 h using LDN57444 (LDN) or DMSO was shown. The CCK-8 assay was used to evaluate survival of H1299 cells and H1299/PEM cells (B), and A549 cells and A549/PEM cells (C) after 48 h of treatment using LDN or DMSO, and the results were evaluated using analysis of variance ( $n = 3$ ). (D) The colony formation assay was performed for A549/PEM-shNC and -sh*UCHL1* cells treated using PEM or

DMSO for 2 weeks, and the results were evaluated using analysis of variance (n = 5).

NS: not statistically significant, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Fig. S4. UCHL1 plays vital roles in progression of the cell cycle in A549 cells and its derived cells.** In the presence of PEM or DMSO, the levels of cell cycle-associated proteins (c-Myc and Cyclin D1) were evaluated using western blot for A549 cells and A549/PEM cells (A), and A549/PEM cells with UCHL1 silencing (C). Flow cytometry was performed to evaluate changes in the cell cycle of A549 cells and A549/PEM cells (B), and A549/PEM cells with UCHL1 silencing (D), with the results evaluated using analysis of variance (n = 5). \* $p < 0.05$ , \*\* $p < 0.01$ .

**Fig. S5. UCHL1 promotes DNA repair through regulating ERCC1.** Western blot showing  $\gamma$ H2AX levels (A) and ERCC1 levels (C) in NSCLC cells that were treated using PEM or DMSO for 24 h (n = 5). (B) The mRNA levels of DNA repair enzymes in NSCLC cells were determined using real-time quantitative PCR (n = 5). (D) Western blot analysis of ERCC1 and  $\gamma$ H2AX levels in A549/PEM-shNC and A549/PEM-sh*UCHL1* cells treated using PEM and DMSO. NS: not statistically significant, \* $P < 0.05$ , \*\* $P < 0.01$ .

**Fig. S6. The mRNA levels and activity of TS in NSCLC cells.** Real-time quantitative PCR analysis of TS (*TYMS*) levels in H1299 and its derived cells (A) and in A549 and its derived cells (B) was shown and the results were evaluated using the Mann-Whitney test (n = 5). The enzyme activity of TS (C) was evaluated in H1299/PEM-sh*UCHL1* cells transfected using either an empty vector lentivirus (-VEC) or *TS*-containing lentivirus (-*TS*), and the results were evaluated using

analysis of variance (n = 5). NS: not statistically significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**Fig. S7. H1299/PEM cells were resistant to PEM in vivo.** The H1299 cells and H1299/PEM cells were subcutaneously injected into BALB/c nu/nu mice, which received weekly intraperitoneally treatments using 100 mg/kg PEM or the vehicle (10% DMSO in PBS). The tumor sizes (A) and body weights (B) were analyzed using analysis of variance (n = 5). (C) Tumor lysates were resolved and the UCHL1 levels were analyzed using western blot (n = 5). (D) The mRNA levels of *UCHL1* were also determined using real-time quantitative PCR (n = 5). \*\*  $p < 0.01$ .

**Fig. S8. The roles of UCHL1 in the PEM resistance of H1299PEM cells in vivo.** The H1299/PEM-shNC cells and -sh*UCHL1* cells were subcutaneously injected into BALB/c nu/nu mice, which received weekly intraperitoneally treatments using 100 mg/kg PEM or the vehicle (10% DMSO in PBS). The tumor sizes (A) and body weights (B) were analyzed using analysis of variance (n = 5). (C) The tumors were removed from the sacrificed mice (upper panel) and the final volumes were evaluated using analysis of variance (bottom panel). NS: not statistically significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**Table S1.** The relationships between UCHL1 levels and clinicopathological characteristics of 220 NSCLC patients

	N	UCHL1 expression		P
		Low	High	
<b>Total cases</b>	220	113	107	
<b>Sex</b>				
Male	108	43	65	<i>P</i> = 0.008**
Female	112	70	42	
<b>Age (years)</b>				
<60	71	32	39	<i>P</i> = 0.1973
≥60	149	81	68	
<b>Tobacco smoking (years × packs)</b>				
≥20 (heavy)	119	54	65	<sup>a</sup> <i>P</i> = 0.0570
<20 (light/never)	24	16	8	
NA	77	43	34	
<b>Pathological TNM stage</b>				
I–II	171	88	83	<i>P</i> = 0.9565
III–IV <sup>b</sup>	49	25	24	
<b>Chemotherapeutics</b>				
Chemosensitive	170	94	76	<i>P</i> = 0.0315*
Chemoresistant	50	19	31	

N, number; NA, not available. Analyses were performed using the  $\chi^2$  test, \**p* < 0.05, \*\**p* < 0.01.

<sup>a</sup> Denotes a significant difference between heavy and light/never tobacco smoking.

<sup>b</sup> Only three patients were pathologically diagnosed with stage IV disease.

**Table S2.** Multivariate analysis of clinical characteristics related to UCHL1 expression

		<b>OR</b>	<b>95% CI</b>	<b>P</b>
	<b>Chemotherapy response</b>			
	Chemosensitive	Reference		
<b>UCHL1 expression</b>	Chemoresistant	2.227	1.136–4.367	0.020*
<b>(high vs. low)</b>	<b>Sex</b>			
	Male	Reference		
	Female	0.392	0.226–0.681	0.001**

OR, odds ratio; CI, confidence interval. \* $p < 0.05$ , \*\* $p < 0.01$ .

**Table S3.** Primers used for the real-time quantitative PCR

Primer name	Sequence (5'- 3')
human <i>UCHL1</i>	CCTGTGGCACAATCGGACTTA CATCTACCCGACATTGGCCTT
mouse <i>UCHL1</i>	AGGGACAGGAAGTTAGCCCTA AGCTTCTCCGTTTCAGACAGA
human <i>GAPDH</i>	GGAAGATGGTGATGGGATT GGATTTGGTTCGTATTGGG
mouse <i>GAPDH</i>	AGGTTCGGTGTGAACGGATTTG GGGGTCGTTGATGGCAACA
human <i>XRCC1</i>	TCAAGGCAGACACTTACCGAA TCCAAGTGTAGGACCACAGAG
human <i>ERCC1</i>	CTACGCCGAATATGCCATCTC GTACGGGATTGCCCTCTG
human <i>MSH2</i>	AGTCAGAGCCCTTAACCTTTTTTC GAGAGGCTGCTTAATCCACTG
human <i>PRKDC</i>	CTGTGCAACTTCACTAAGTCCA CAATCTGAGGACGAATTGCCT
human <i>TYMS</i>	CTGCTGACAACCAAACGTGTG GCATCCCAGATTTTCACTCCCTT
mouse <i>TYMS</i>	GATTCAGATTACTCGGGACAAGG CAGAGCATAGCTGGCAATGT

*UCHL1*, ubiquitin C-terminal hydrolase L1; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *XRCC1*, X-ray repair cross complementing 1; *ERCC1*, excision repair cross-complementing 1; *MSH2*, mutS homolog 2; *PRKDC*, protein kinase, DNA activated, catalytic polypeptide; *TYMS*, thymidylate synthetase.

**Table S4.** The relationships between chemotherapy regimens and responses in 63 NSCLC patients

	N	Chemotherapy response		<i>P</i>
		Chemo-sensitive	Chemo-resistant	
<b>Total cases</b>	63	32	31	
<b>Containing pemetrexed (with platinum)</b>	36	21	15	0.1669
<b>Without pemetrexed (platinum plus paclitaxel [14], plus gemcitabine [8], plus vinorelbine [5])</b>	27	11	16	

N, number. Analyses were performed using the  $\chi^2$  test, \*  $p < 0.05$ .

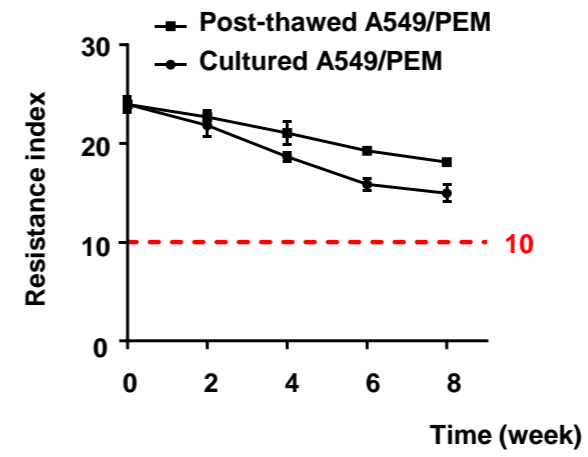
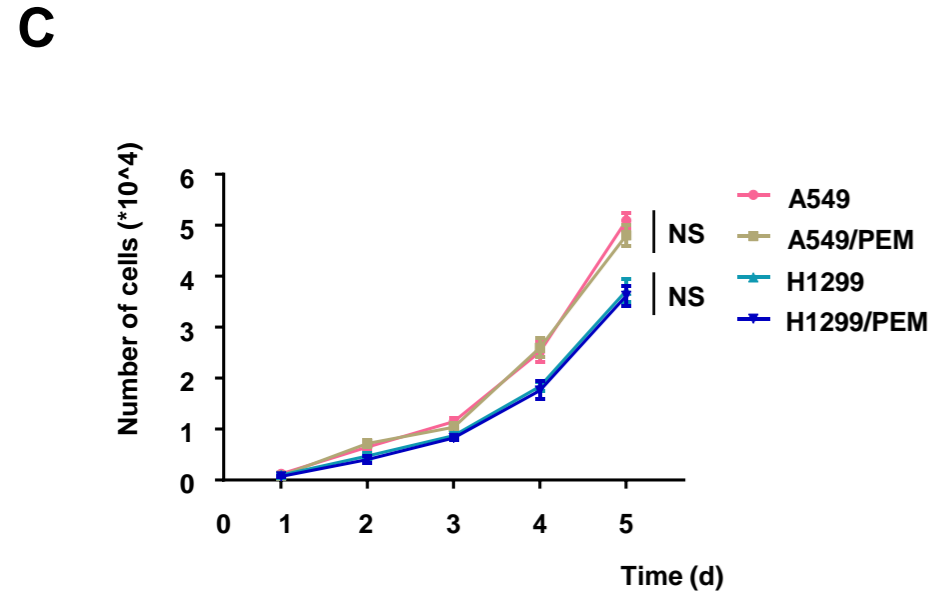
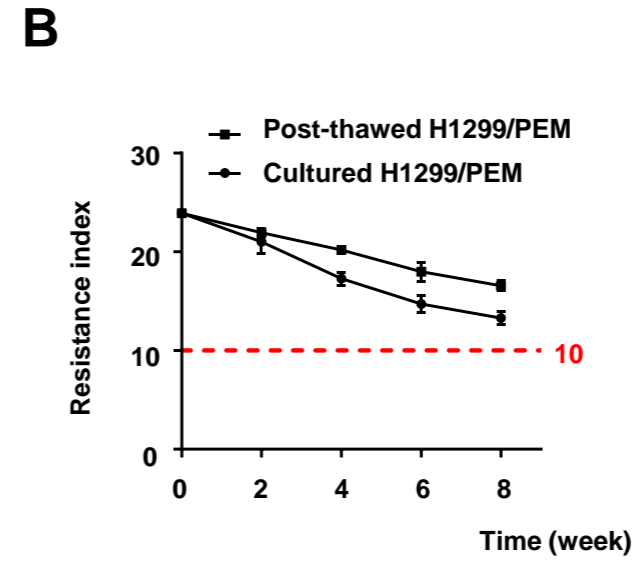
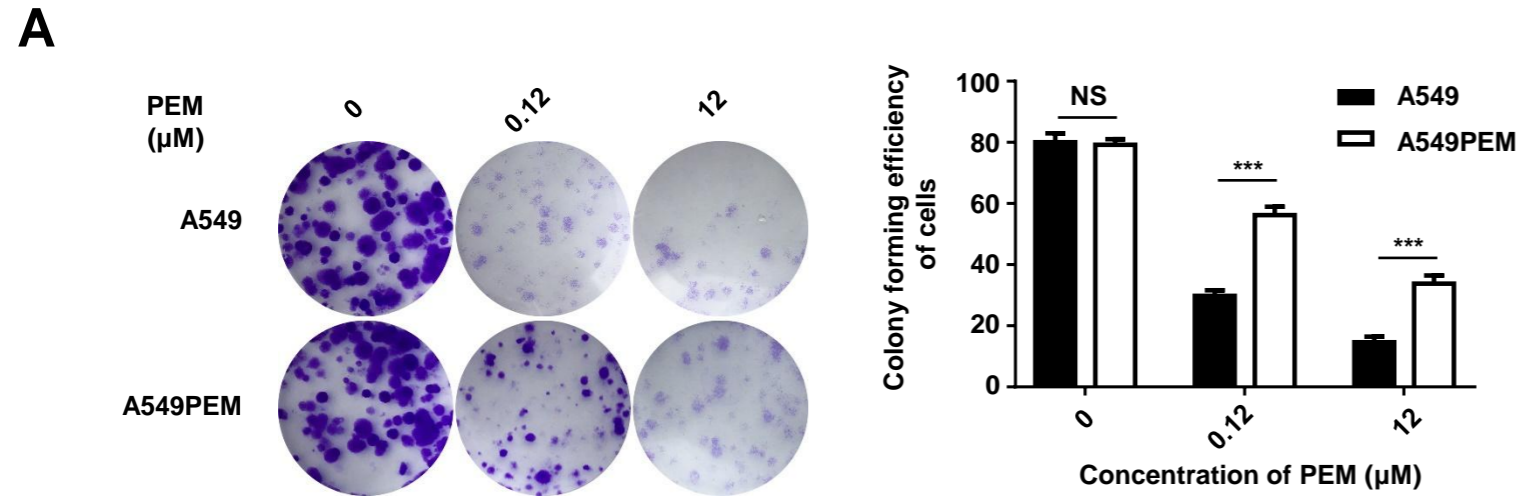
**Table S5.** Multidrug sensitivities of the two PEM-R NSCLC cell lines and their parental cell lines

Drug	IC50		Resistance index	<i>p</i>	IC50		Resistance index	<i>p</i>
	H1299	H1299/PEM			A549	A549/PEM		
Pemetrexed	0.66±0.13 (μM)	14.33±1.74 (μM)	23.99±3.80	0.0079**	1.15±0.23 (μM)	25.28±4.42 (μM)	23.51±2.90	0.0079**
Taxel	1.92±0.37 (nM)	18.24±4.60 (nM)	9.66±1.59	0.0079**	3.18±0.35 (nM)	4.77±0.46 (nM)	1.62±0.30	0.0556
Gemcitabine	0.13±0.03 (μM)	0.33±0.09 (μM)	3.26±1.02	0.0952	2.54±0.80 (μM)	4.67±0.75 (μM)	2.42±0.75	0.0556
5-fluorouracil	3.72±1.04 (μM)	46.30±5.68 (μM)	15.43±3.20	0.0079**	2.00±0.09 (μM)	40.59±2.52 (μM)	20.16±1.68	0.0079**
Docetaxel	1.84±0.77 (nM)	2.49±0.85 (nM)	2.08±0.74	0.3095	1.93±0.41 (nM)	9.29±1.35 (nM)	4.12±1.17	0.0317*
Carboplatin	8.10±0.96 (μM)	65.12±4.81 (μM)	8.62±1.39	0.0079**	12.20±0.94 (μM)	135.78±6.49 (μM)	11.47±1.25	0.0079**
Cisplatin	1.01±0.19 (μM)	13.86±2.64 (μM)	14.23±2.16	0.0079**	0.79±0.13 (μM)	10.07±2.17 (μM)	13.28±2.30	0.0079**

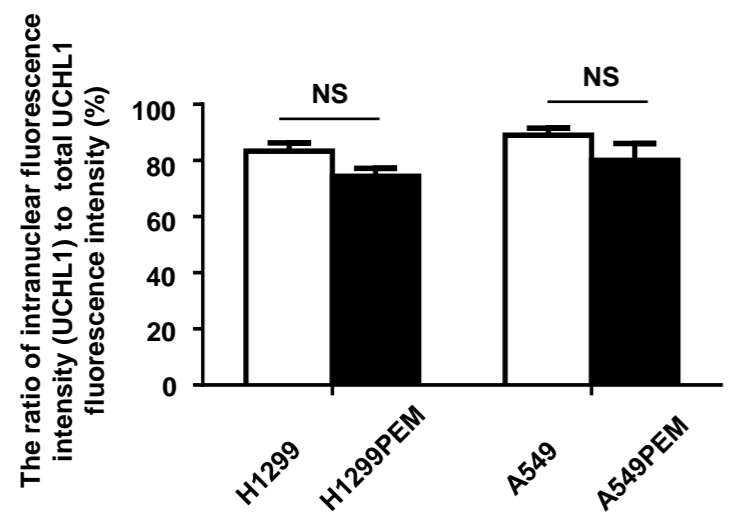
IC50: 50% inhibitory concentration. Sensitivities of the NSCLC cells to the drugs were determined using the CCK-8 assay. The resistance index represents the ratio of the IC50 in the PEM-R cell to the IC50 in the parental cell for each drug. Statistical analyses were performed using the Mann-Whitney test ( $n = 5$ ), \*  $p < 0.05$  or \*\*  $p < 0.01$ .



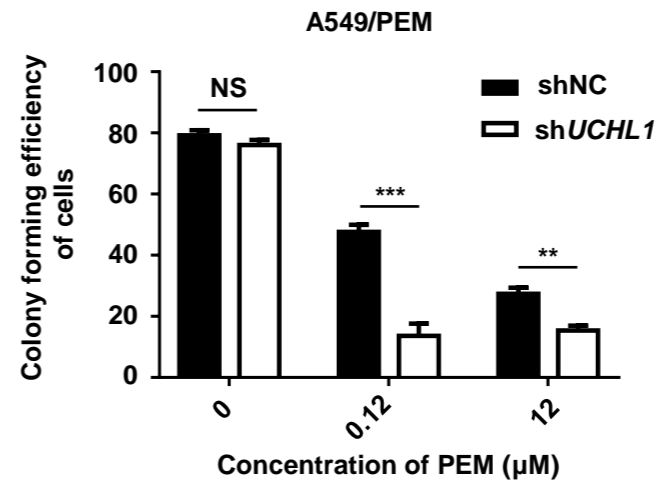
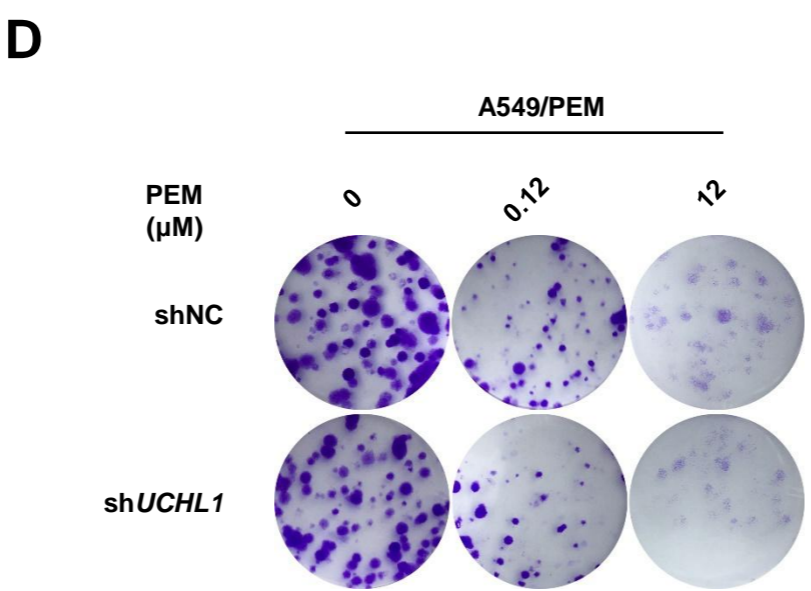
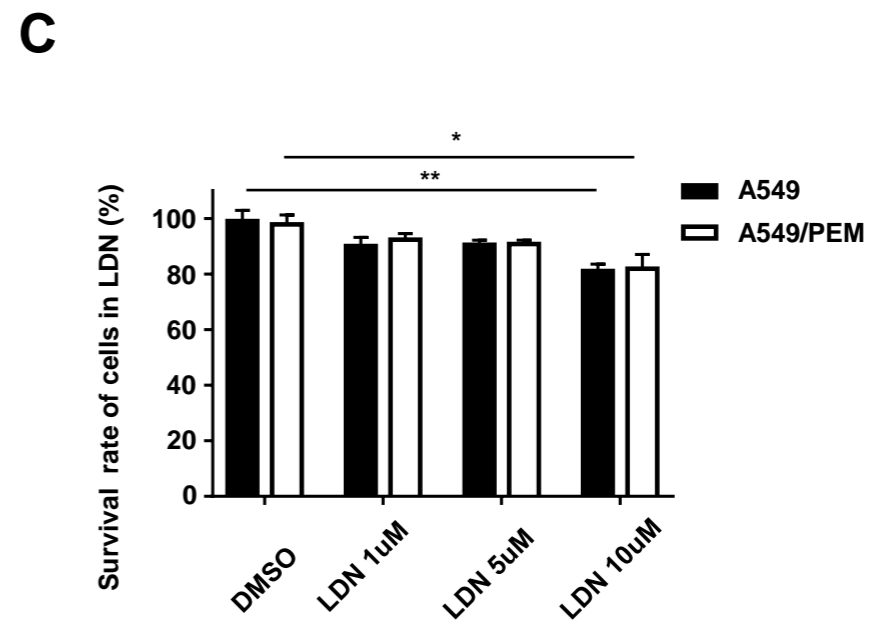
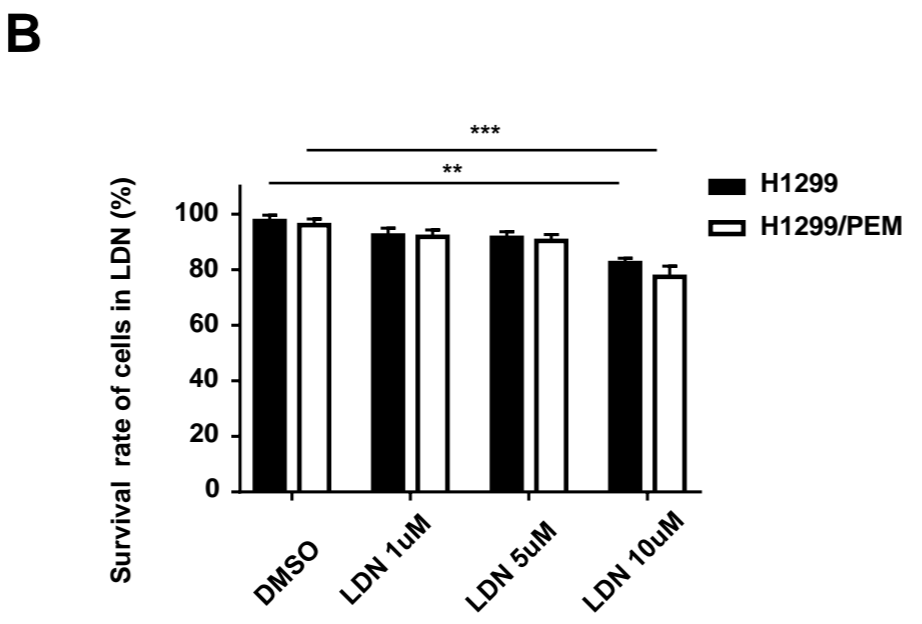
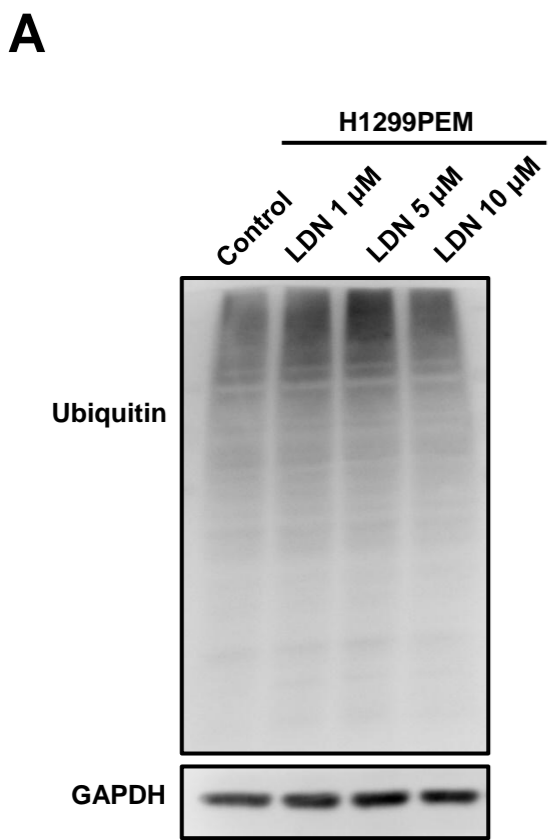
# Supplement figure 1



# Supplement figure 2

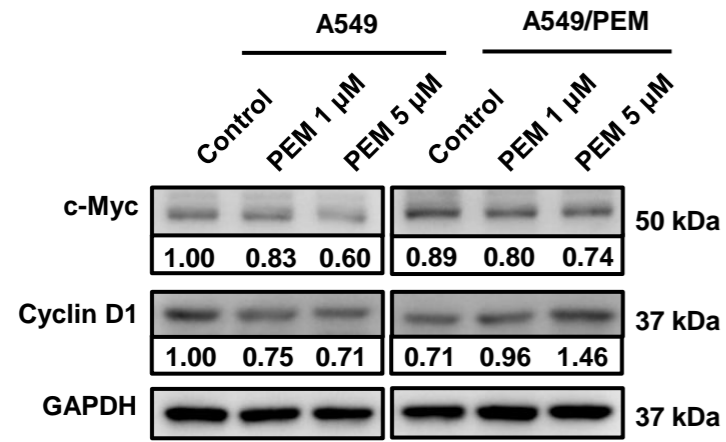


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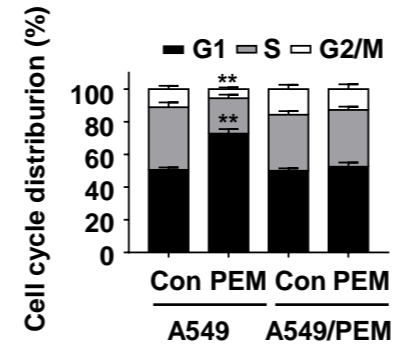
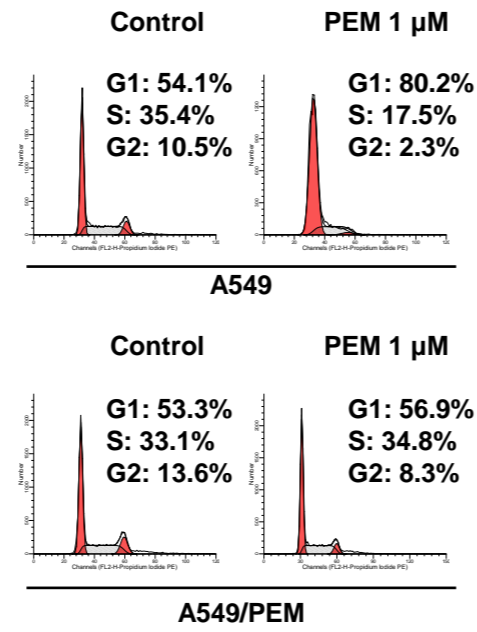


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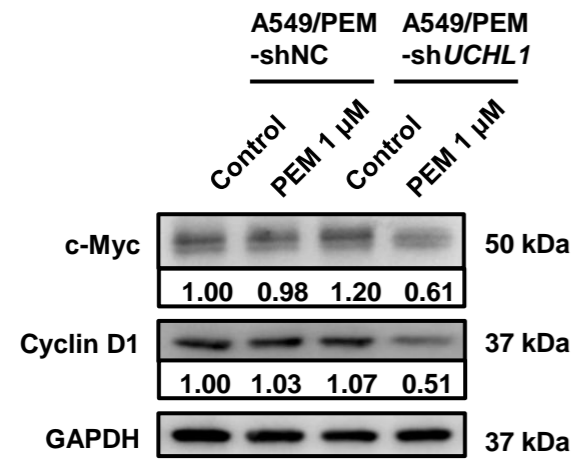
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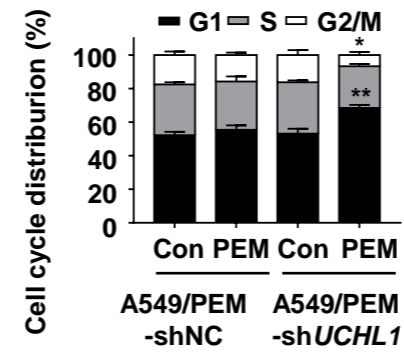
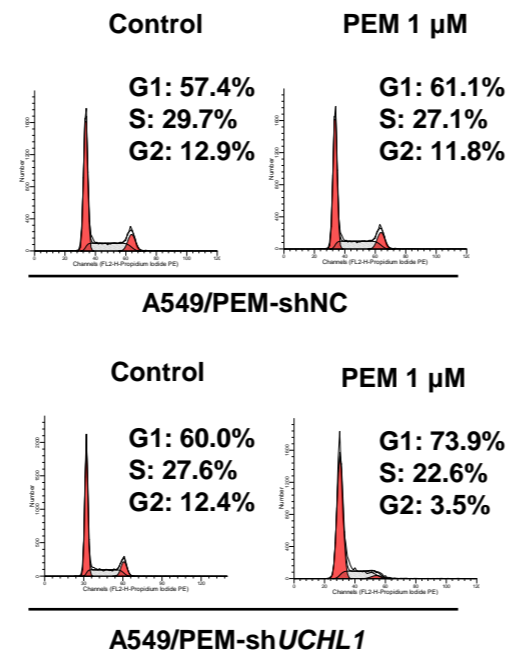
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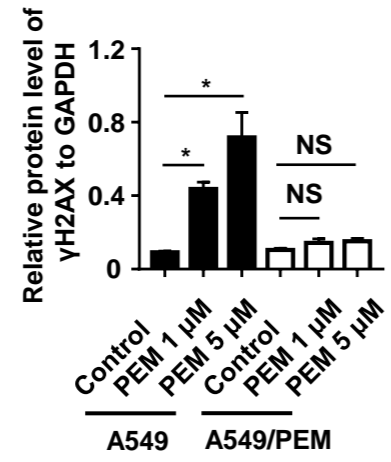
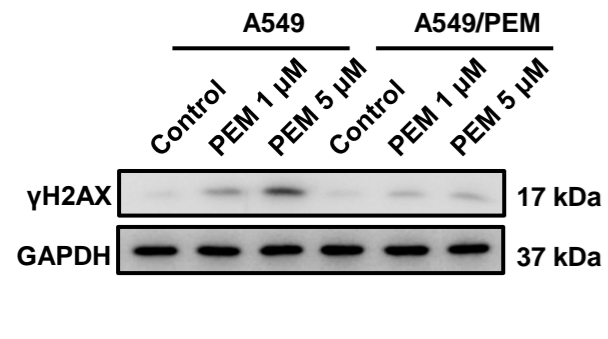


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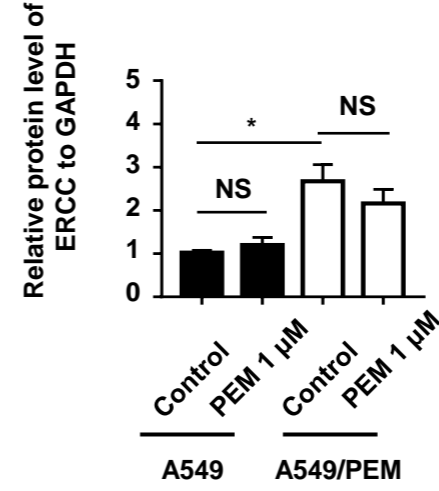
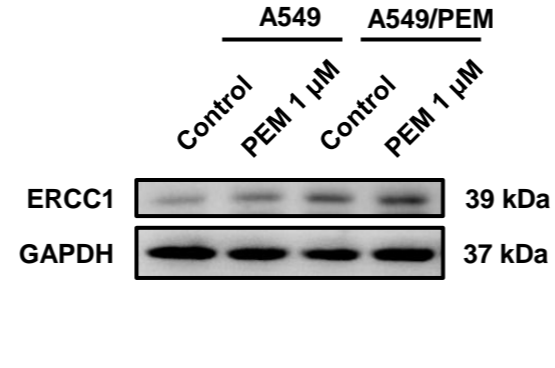


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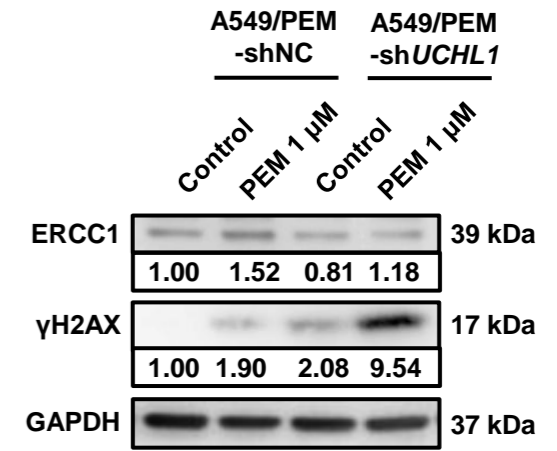
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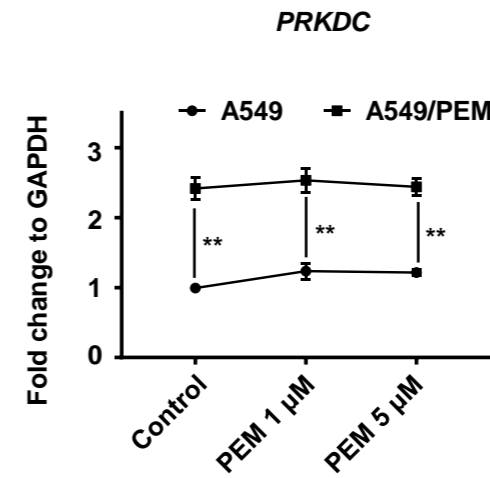
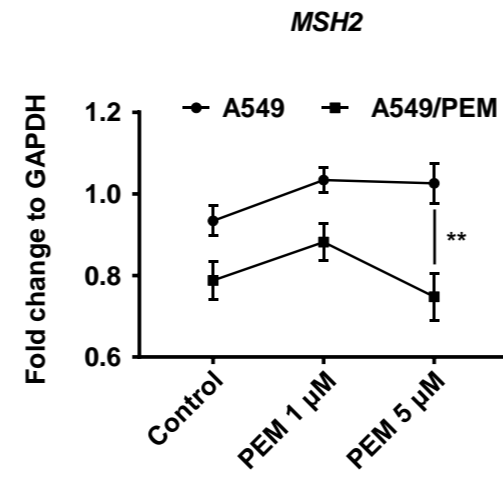
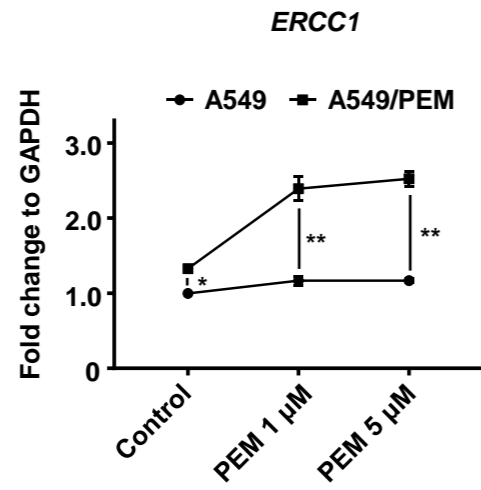
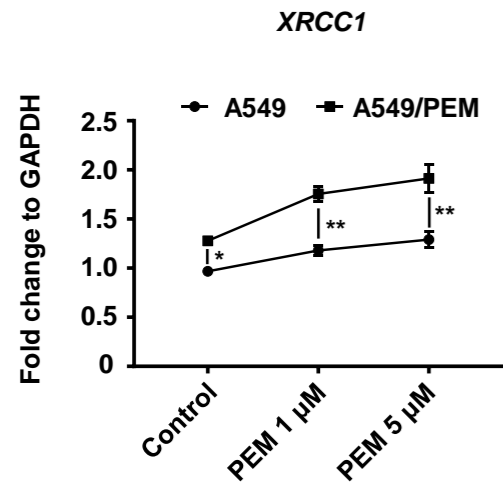
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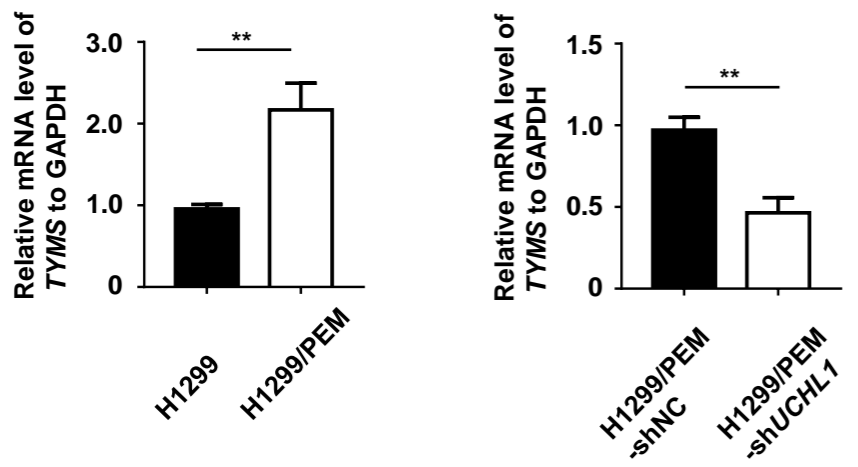


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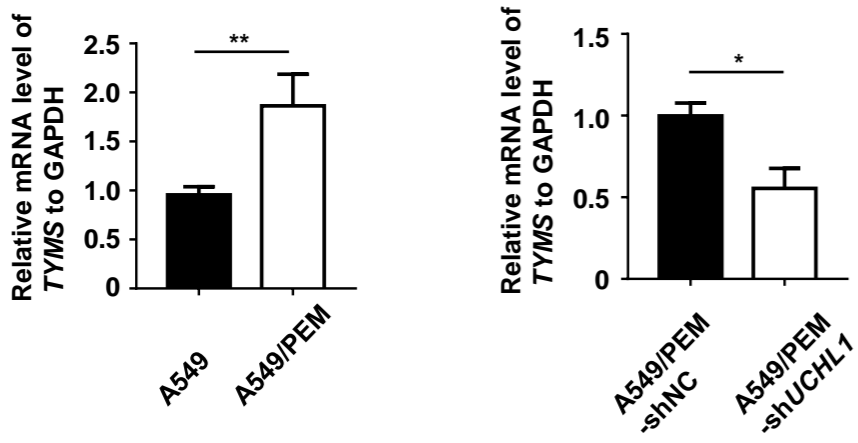


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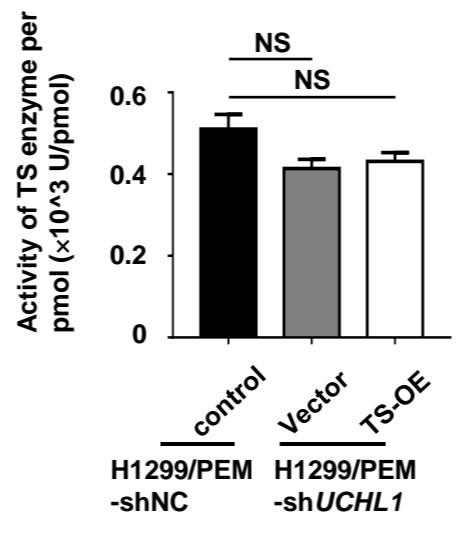
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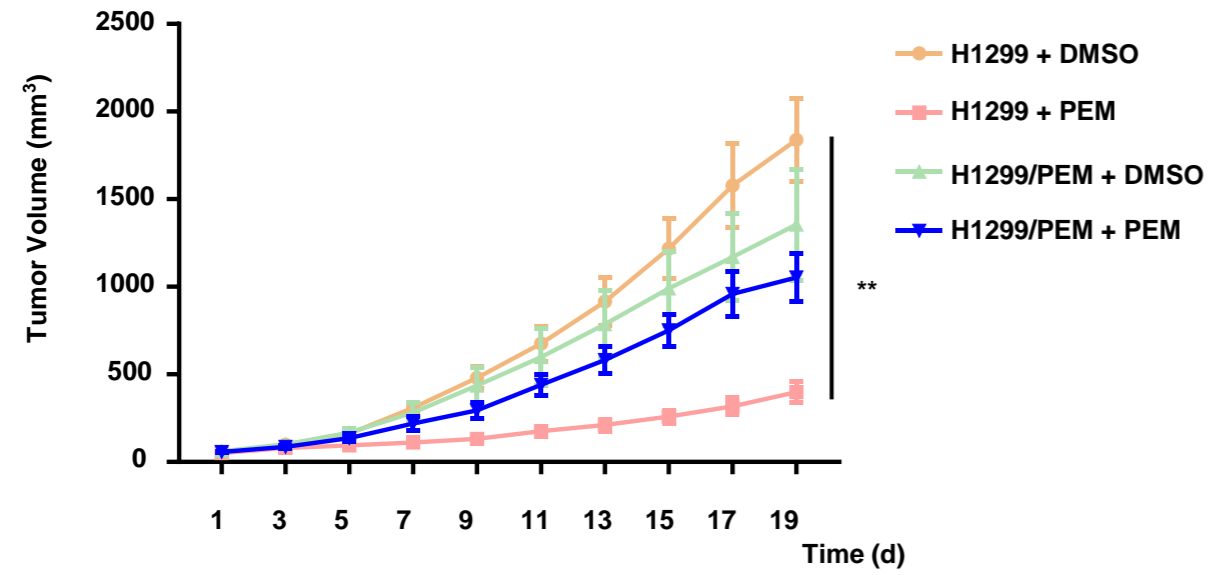


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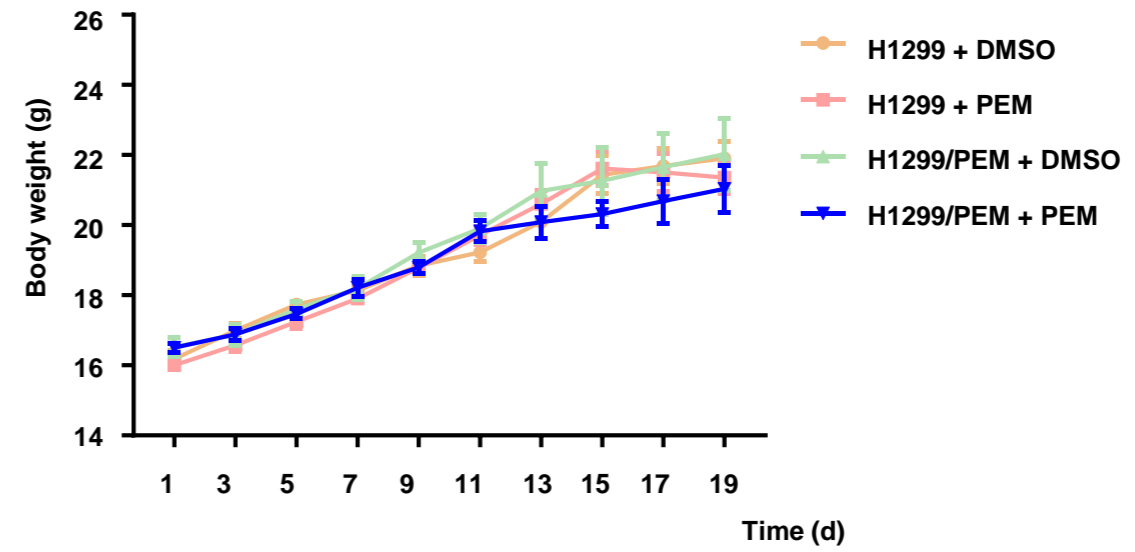


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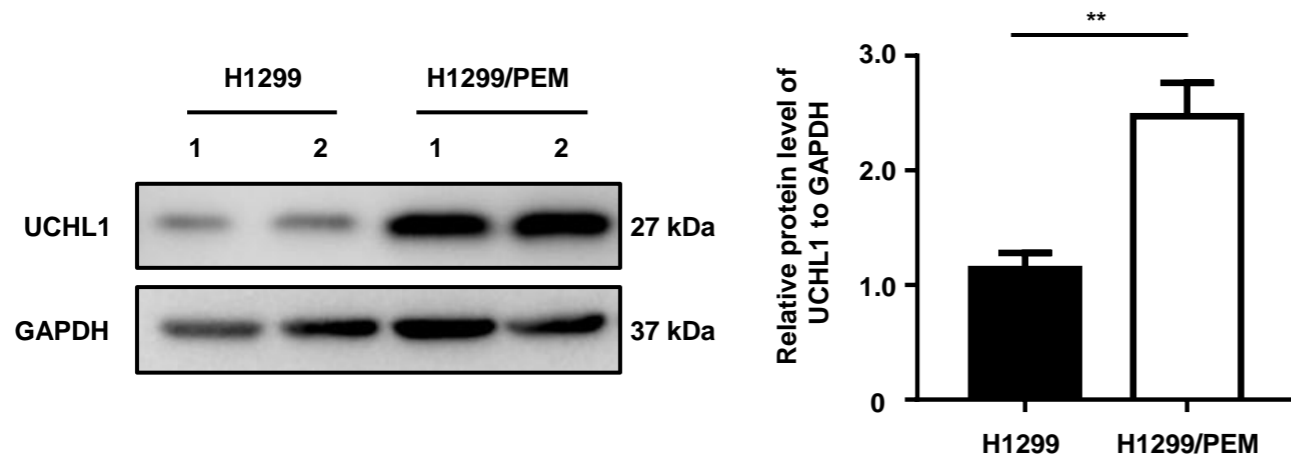
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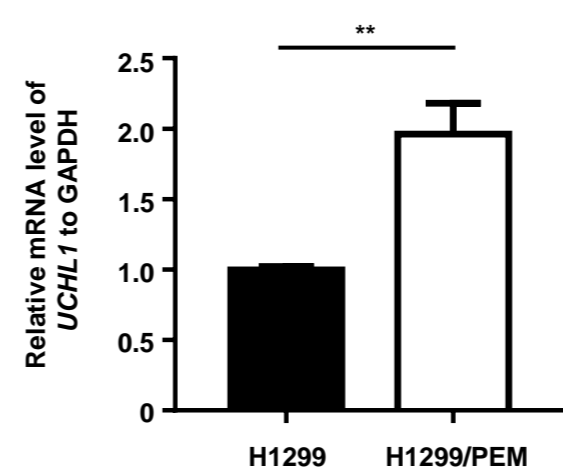
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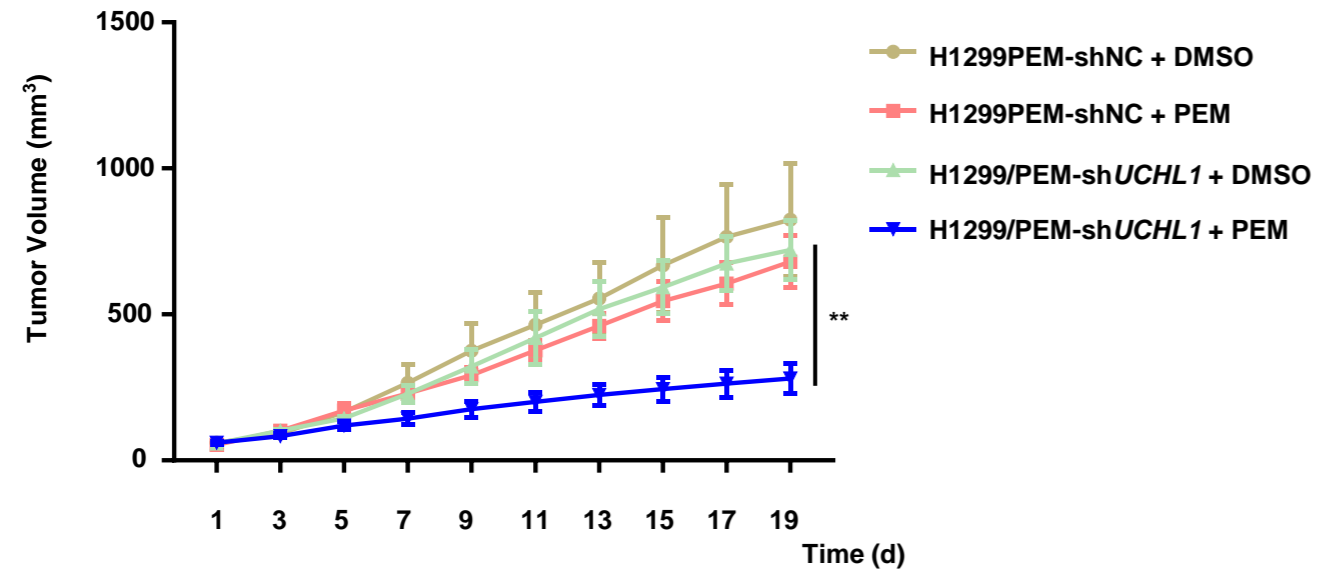


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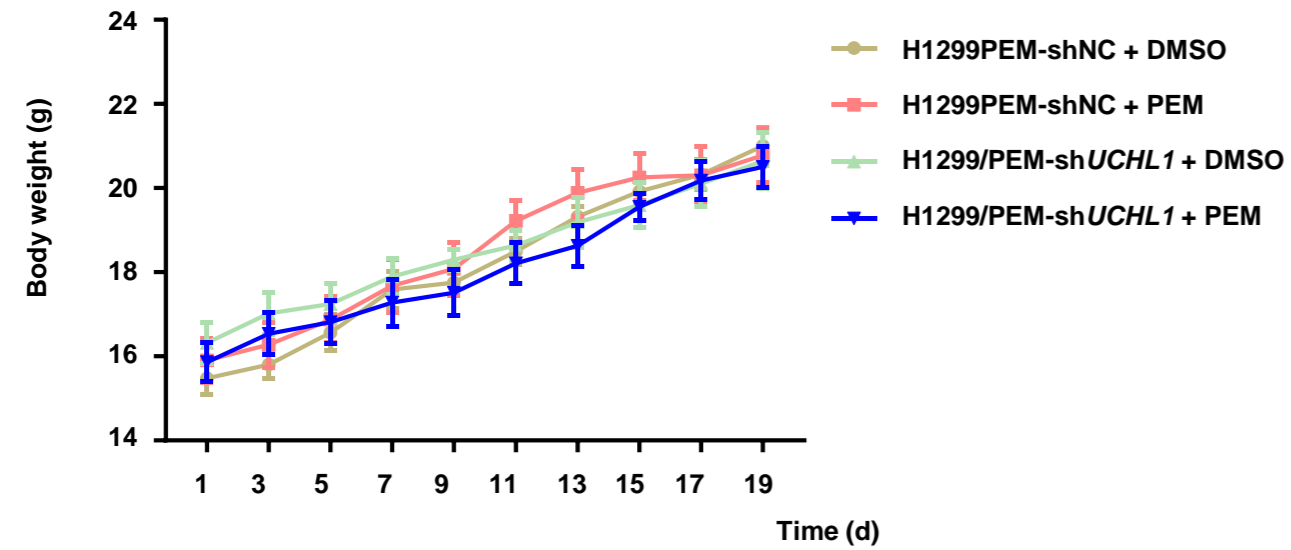


# Supplement figure 8

**A**



**B**



**C**

